Characterization and Biocompatibility Study of Nematic and Cholesteryl Liquid Crystals

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***Abstract*— Intensive research in bio-engineering has been conducted in the search for flexible biomaterials that could support cell growth and cells attachment. Flexible synthetic materials that support cell growth without the aid of synthetic extracellular matrix proteins are still rare. Cholesteryl liquid crystal containing cholesteryl moieties may have suitable biological affinity. Human keratinocytes (HaCat) were cultured with a nematic liquid crystal and three cholesteryl liquid crystals of different formulation. Subsequently, the trypan blue dye exclusion assay was used to determine cell viability in the liquid crystals. The two classes of liquid crystal were characterized by Differential Scanning Calorimeter (DSC) and polarizing microscope (POM) to understand the nature of the interface material. The cell viability study in medium containing liquid crystals verified that liquid crystals had no effects on cell viability. However, only the surface of cholesteryl liquid crystal has shown affinity to HaCat cells. In addition, cells continued to proliferate in the presence of liquid crystals without a change of medium for eight days. No sign of exothermic and endothermic activities at 37^0C were observed from the DSC test results for the three samples. Biological and mechanical test result of the cholesteryl liquid crystals has shown that cholesteryl liquid crystals are non toxic and support cell attachment without extracellular matrix protein at very low elasticity.**

***Index Terms*— Cell adhesion, Cell viability, Human keratinocytes, Liquid Crystals.**

I. Introduction

The exploitation of liquid crystals as biomaterials are attracting great interest in the area of biomedical engineering because of liquid crystals may provide a means of label-free observations of biological phenomenon [1]. Using liquid crystal as biosensor to probe mechanical response of mammalian cell is still in the infancy stage. Before applying liquid crystals to optically probe biological systems with success, some key issues need to be resolved. Biocompatibility of liquid crystal is the entry requirement for fabricating liquid crystal as biosensors for the cells-surface interfaces. A few liquid crystals of cyano, imine and ether functional groups have been identified to be cytotoxic, e.g. 5CB, E7, "A", "B" series liquid crystals are toxic to 3T3 fibroblasts and SV-40 HCECs [2,3]. In the same study, it was revealed that cells are viable on a mixture of molecules of diflurophenyl and bicylohexane groups such as "C" series and TL205 liquid crystal. Consequently, these results were applied to reveal the capability of new method of culturing cells through TL205/matrigel and imaging the reorganization of matrigel by HCEC cells [4]. In this work, ester based cholesteryl liquid crystals are explored for the development of bio-transducer. Initial study of cell viability and characterization of cholesteryl liquid crystals will be reported in this paper.

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II. Experimental

A. Liquid crystal preparation

Shear sensitive cholesteryl liquid crystals were synthesized according to the procedure given in [5].The cholesteryl liquid crystals purchased from Aldrich consisted of Cholesteryl chloride $C_{27}H_{45}Cl$, Cholesteryl Perlargonate $C_{36}H_{62}O_2$ and Cholesteryl Oleyl Carbonate $C_{46}H_{80}O_3$. The melting points for these liquid crystals are 94^0C-96^0C, 74^0C-77^0C and 113^0C, respectively. Three liquid crystal samples of different formulations were synthesized from these liquid crystals. They were termed as CLCP1 (12% $C_{27}H_{45}Cl$, 44% $C_{36}H_{62}O_2$, 44% $C_{46}H_{80}O_3$), CLCP2 (25% $C_{27}H_{45}Cl$, 38% $C_{36}H_{62}O_2$, 38% $C_{46}H_{80}O_3$) and CLCP3 (50% $C_{27}H_{45}Cl$, 25% $C_{36}H_{62}O_2$, 25% $C_{46}H_{80}O_3$). The melting points for these liquid crystals are 94^0C-96^0C, 74^0C-77^0C and 113^0C, respectively. Nematic liquid crystal TL205 was purchased from Merck, Japan and with a clearing point of 87.4^0C.

B. Cell culture

Cells for this experiment were obtained from sub-cultured human keratinocytes (HaCat) in a $25cm^2$ cell culture flask. First, old medium of the culture flask with confluent cells were removed from the cell culture flask and the flask were washed three times with Hanks Balance Salt Solution (HBSS). After removing the HBSS solution, 1ml of trypsin (0.5mg/ml) was pippetted into the flask and it was incubated for about 4 minutes in a humidified 4% CO_2 atmosphere. Subsequently, the flask was examined under the phase contrast microscope to ensure that all the cells were

detached from the surface of the cell culture flask. 5ml of RPMI 1640 Medium were then deposited to halt the trypsinisation process. The cell suspensions were transferred to the centrifuge tube and centrifuged for 5 minutes at 1600rpm. When this process is completed, supernatant were discarded and the cells were re-suspended in 6ml of culture medium. Eventually, this cell suspension was ready to be plated in the petri dish or culture cluster containing the liquid crystal cover slip.

C. Cells viability study

Nematic, TL205 and three cholesteryl liquid crystals (CLCP1, CLCP2 and CLCP3) were used in the study of cell viability. The four liquid crystals and negative control were prepared for this study in a 12 well cell culture clusters. Cell lines of Human keratinocytes were obtained with the standard sub-cultured procedures and 5ml of cells suspension at 16 x 10^4 cells/ml cell density was prepared. One ml per well of cells suspension were plated in the negative control well and 4 wells containing the test specimen. 2ml of HEPES containing medium was added to each well. Prior to the plating of cells, 5µl of cholesteryl liquid crystals were pipetted in each well. The cells culture plate was tightly sealed and incubated at 38^0C for 24 hours. On the following day, the medium and liquid crystals of every well were removed from the cell culture cluster and 1ml of trypsin was added to each well containing the samples. Subsequently, after about 4 minutes, 2ml of medium was added to halt the trypsinisation process. The dye exclusion assay with 0.4% of trypan blue was used to determine the stained and unstained cells for each sample in the plates. 0.1ml of cells sample and 0.1ml of trypan blue was sampled and mixed in a 15ml test tube and counted ten times on a haemocytometer in three separate experiments. Percentage of for each treatment was calculated based on viable cell count/total cell count x 100%. Mean values of the measurements are expressed as Mean ± standard error of the mean. Analysis of variance using one way ANOVA followed by Turkey's HSD test was used to determine the statistical differences between means and the control.

D. Characterization of the liquid crystals

A temperature stability study was conducted for the three liquid crystals by using crossed polarizing microscope and differential scanning calorimeter. Under the illumination of AxioPlan2 polarizing optical microscope (POM) at 40x magnification, each sample was in turn placed on Linkam THM600 hot stage which was controlled by Linkam Control plug-in in AxioVision software. Subsequently, ramp of heat were applied to the samples at 1^0C/min and a sequence of transition images were captured at 1 frame/sec. The simulation temperature was terminated at 37^0C. Further thermal analysis on the samples was carried out with Differential Scanning Calorimeter (DSC). For the DSC analysis, 2-3mg of each sample was analyzed under DSC Q2000. Ramp temperature of 1^0C/sec was applied to the samples from -50^0C to 120^0C. The relationship was obtained in terms of heat flow and heating temperature.

III. Result and Discussion

A. Cell viability study

The cells viability study was used to determine the toxicity of the liquid crystals. The survival rate provides an indication of toxicity of the liquid crystals tested. The outcome obtained in Fig. 1 indicated that both nematic and cholesteryl liquid crystals were not toxic to HaCat cells and cells had viability greater than 93% (Fig. 1). No significant differences in cell viability were identified for the CLCP1 ($p=0.993$), CLCP2 ($p=0.524$),CLCP3 ($p=0.980$) and TL205 ($p=0.992$) compared with the control (Turkey's HSD test, $P>0.05$). This shows that the liquid crystals used are non toxic to HaCat cells. This result is comparable with the percentage of cell viability reported in [2] for cholesteryl and TL205 liquid crystal. In contrast to [2], this work did not find TL205 to support higher cell viability than the cholesteric liquid crystal as reported. Comparison is unjustified because Abbott et. al. did not specify which type of cholesteryl liquid crystal was used in their work. Cholesteryl liquid crystal has shown affinity for cell attachment without any use of extracellular matrix protein to aid adhesion. Unfortunately, this was not observed for TL205 in which the surface seemed to be less viscous (367mPa) compared to the cholesteryl liquid crystal (Fig. 2). Similarly, it was found in coherence with [4] that no cells attachment was found on TL205. Nematic liquid crystal such as TL205 requires extracellular matrix proteins, i.e. matrigel, fibronectin or laminin to aid cell attachment and may not be a suitable candidate for this work. If extracellular matrix proteins were to be used, cells might react to the ligands density rather than interfacing directly to the material itself [4]. However, cells proliferated at the surrounding of the liquid crystal drop. The cholesteryl liquid crystal might contain cholesteryl moieties which support cell adhesion directly on the material because synthetic cholesteryl esters liquid crystal had similar chemical structures to cholesteryl esters in a lipid core [6,7] and thus serve as a bio-compatible amphiphile layer mimicking a single lipid layer of a cell membrane. Furthermore, these ester based liquid crystal used are also found in the cosmetic formulation [8]. Instead of a drop of liquid crystal as used in the viability study, cholesteryl liquid crystal was spread evenly on the cover slip and plated with cell suspension at 5 x 10^4 of cell density and incubated at 37^0C. A large colony of HaCat cells was attached and buckled the surface of the three types of liquid crystal (Fig. 3A). As the cell density was lowered to 2 x 10^4, individual cell adhesion could be observed (Fig. 3B). Hyper-proliferative HaCat cells on the liquid crystals that function as cells force transducer might be useful for the assessment of pharmacological agent which influence cell proliferation. For example, ripovine and lidocain were used to study their effects on the proliferation of human keratinocytes [9]. In addition to this study, cell force transducer could provide additional information on the cell mechanic studies in response to these pharmacological agents. The study on elastic property in has shown that the cholesteryl liquid crystal has linear elastic property when sheared in the range of $0.1s^{-1}$ to $1s^{-1}$ but behaved as pseudo-plastic beyond $1s^{-1}$. Human keratinocytes movements most probably occur within the linear elastic range of the cholesteryl liquid crystal.

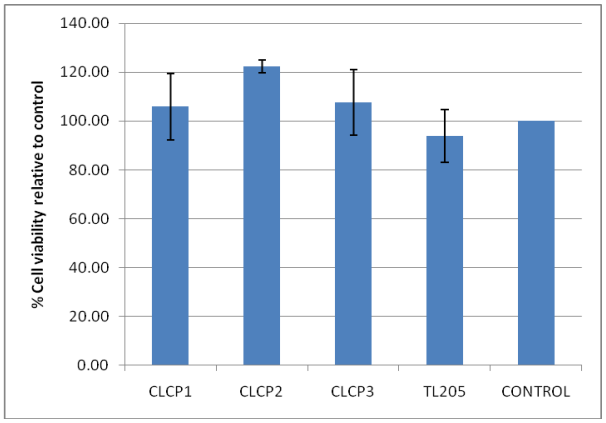


Fig. 1. Percentage of cell viability relative to control in the presence of cholesteryl and nematic liquid crystal.

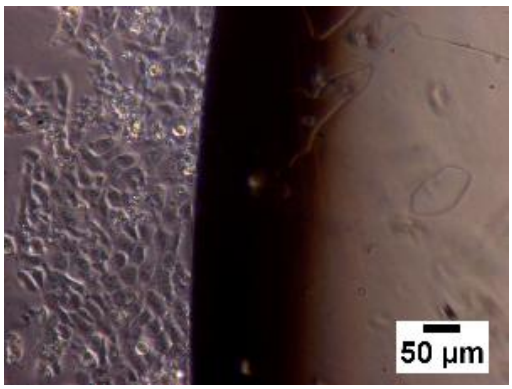


Fig. 2. Culturing HaCat cells in the presence of TL205 liquid crystal. The surface did not support direct cells attachment but this liquid crystal shown non-toxicity to cells.

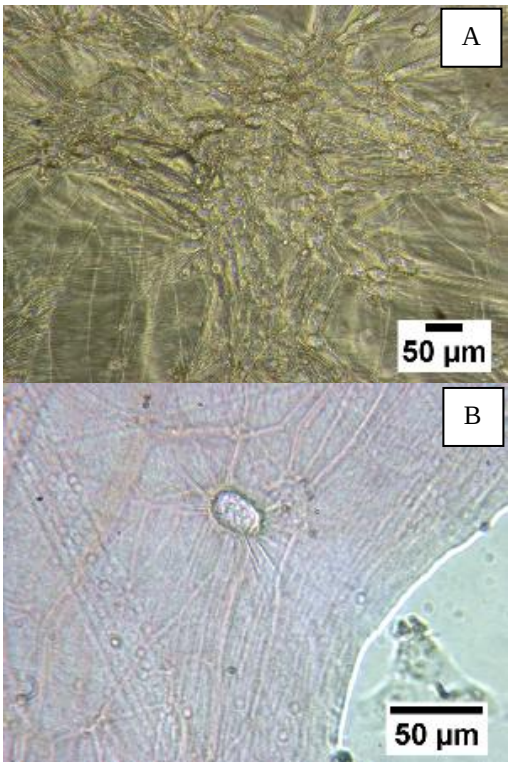
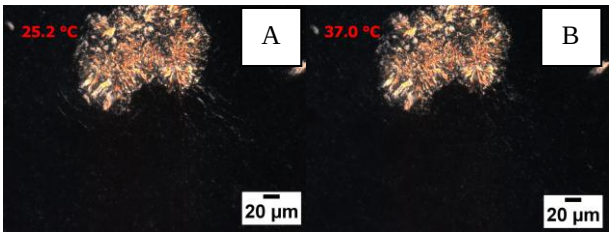


Fig. 3. HaCat cells adhesion to cholesteryl li quid crystal (CLCP2). (A) Colonies of HaCat cells attached and wrinkled the surface of the cholesteryl liquid Crystals. (B) A single HaCat cell translated the mechanic adhesion to buckling effects on the cholesteryl liquid crystal (CLCP2).

B. Temperature stability of the liquid crystals

The temperature response of both liquid crystal types was studied qualitatively. Under the illumination of the polarizing microscope, the three cholesteric liquid crystals samples were sheared by a cover slip and birefringence property of the material was greatly reduced with mono optical reflection. Other than the defect oil streaks lines (Fig. 4A and Fig. 4B), the orientation of the mesogens were in the isotropic orientation under the cover slip compression because they are shear sensitive and have underwent a pitch change. After heating the samples on the hot stage, the major structures of the CLCP1, CLCP2 and CLCP3 under test remained unchanged from room temperature, 26^0C to simulated incubation temperature, 37^0C. However, the color reflection of CLCP1 and CLCP3 had undergone a slight variation. Minimum phase change on the liquid crystal is expected when these samples are incubated. This is probably due to the combination effects of the three compounds providing optimum temperature sensitivity to the three cholesteryl liquid crystals [5]. In contrast with the cholesteryl liquid crystals, TL205 maintained the birefringence property and mesogens were maintained in the homeotropic state even though compressed by the cover slip. Heat ramp from room temperature to incubation temperature (37^0C) did not show much effect on the structure of the nematic liquid crystal as shown (Fig. 4C and Fig. 4D). This result is expected because the nematic liquid crystals are used in liquid crystal displays and they are meant to be sensitive to electrical potential and stay stable at room temperature in an electronic product.

Thermal analysis using Differential Scanning Calorimeter (DSC) was carried out with great sensitivity to detect energy changes or heat capacity changes. However, only the result for cholesteryl liquid crystals is shown here. The result were expressed in heat flow as a function of temperature. From the DSC curves in Fig. 5 for CLCP1, CLCP2 and CLCP3 samples, one could observe that the glass transition temperatures (T_g) appeared as steps in the baseline due to an exothermic reaction and that T_g occurs at ~ -38.67, -34.26 and -28.75°C for CLCP1, CLCP2 and CLCP3 compositions, respectively. As the temperature increased, each sample reached its first melting temperature (T_m) which can be seen as endothermic peaks / reactions at temperatures ~ 51.43, 54.83 and 58.33°C for CLCP1, CLCP2 and CLCP3, respectively (see Fig. 5). At the desired temperature range between 26^0C to 37^0C, all three samples remain chemically stable between just above T_g and up to first T_m, i.e. there were no sign of endothermic or exothermic activity in the range -20°C to +50°C. All three compositions, therefore, have unique formulation that allows staying stable over a wide temperature. This would actually enable their uses in monitoring cell surface interracial forces.



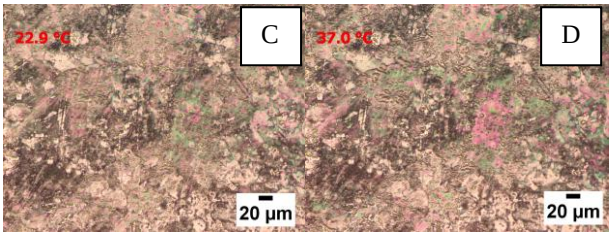


Fig. 4. Effects of temperature to the phase transition of the (A & B) cholesteryl liquid crystals CLCP2 and (C & D) nematic liquid crystal. Isotropic phase of cholesteryl liquid crystal, CLCP2 at (A) room temperature and (B) incubation temperature. Nematic phase of TL205 at (C) room temperature and (D) incubation temperature.

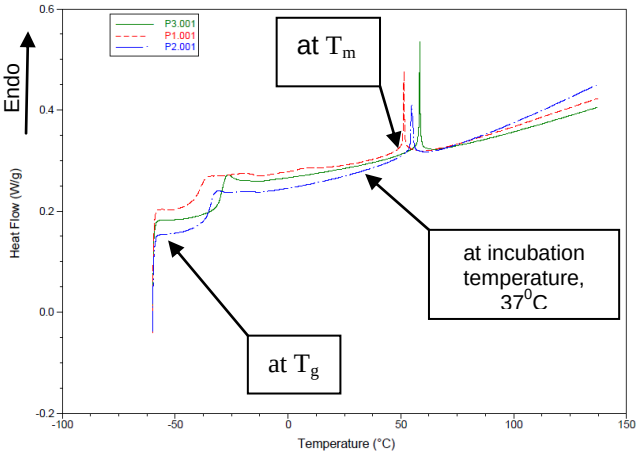


Fig. 5. DSC shows heat flow as a function of temperature for the three compositions of cholesteryl liquid crystals. No exothermic or endothermic activities were observed at 37^0C.

IV. CONCLUSIONS

Nematic and cholesteryl liquid crystals are biocompatible with human keratinocytes. However, the results reported in this paper suggest that TL205 might not be suitable as an interfacing layer for the direct monitoring of cell/surface without coating of adhesion ligands. On the other hand, the cholesteryl liquid crystals enabled cell adhesion and proliferation without the need for pre-treatment with extracellular matrix molecules. A single and colonies of cells were achieved by controlling the density of cells plated. The cholesteric liquid crystals were found stable without phase change and heat flow at room and incubation temperatures. The cells force transducer developed has great potential to be used in toxicology and pharmacology assessment.

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